

S51. ✓ 0 glut prol orn citr arg. E-arg. Ho prol ^E arg N
 +++ +++ - ++ ± ~~+++~~ - +++ +++
 later, all up - adapted (!?)

S55 ✓ 0 - trypt T+ cyst T+ meth T+ S-AcOH.
 ++ +++ +++ +++

S52. ✓ NV E- all +++ exc. trypt. ++.

S50. ✓ Biotin + N- all - BN, BE++ !!

S57. ENV EN NV EV E N V O
 - ~~+++~~

S58 ENV EN NV EV E N V O
 +++ ++ + + - ± - (may have adapted
 in slant (large +
 small colonies on
 Y slant).

S60 " " " " " " " +++

S56. ✓ 0. ~~trypt.~~ Biotin l-tyrosine dl-tyrosine. diolol
 - +++ +++ +
 (looked like
 a contamin.)

l-tyr. l-Pal gly-tyr (tyr-gly) glut-tyr trypt trypt gly delucides POH=O POH=glyc glyc de-
 +++ - +++ - +++ +++ - cell - - hydrol glyc.

chudam alkohol.

	mg per 5cc
Blank	
L-tyrosine	0.10
L-phenylalanine	0.10
glycyl-L-tyrosine	0.16
L-tyrosyl glycine	0.15
L-glutamyl-L-tyrosine	0.25
L-tyrosyl-L-tyrosine	0.20
glycyl dehydro phenyl alanine	1.5

filtered solutions

p-OH phenyl pyruvic	1.0
* phenyl pyruvoyl glycine	1.5
* glycyl dehydro phenyl- alanyl glycine	1.5

* old solutions.

29 OCT 1946

washed suspension 10^9

Irradiate 410 1, 2, 5 mins.

SP 10/28.

incub. 2 ml into 10 ml YB cholate

11A29. Spread

5 min. ca 100 cells on L-endo-lactose + EMB-lactose plates. incubate at 30°.

~~no~~ no mutants / 3000 on EMB.

Inc 1 ml 2 min. culture.

Re-irradiate 1, 2, 5.

spread on ~~100 plates~~ (EMB-lactose).

found 2 mutants:

161 x ~~||||~~ =

64 ||

450 x ~~||||~~ ||

200

75 ||

ca 200/plate average.

$\frac{80}{16,000}$

6 pink colonies which are not obviously contaminants.

Plate complete for further identification.

- 1 yeast - cont
- 2 morph. typical coliform.
- 3 came up v. slowly on YB agar
- 4 yeast cont.
- 5 yeast cont.
- 6 came up v. slowly on YB agar.

∴ 2 is only likely possibility of a lactose - coli mutant:

335-2:

T₁^S

requires T, L, B. ✓

Y 53

Activity on various sugars: K-12, Y53. 335 r

11/13/46

The strains were tested on EMB plates & sugars:

glucose sucrose maltose lactose gelactose

K-12 +✓ -± + + +

Y53. +✓ -± + - + { this is interesting! }

Salmonella crosser.

28 OCT 1946

Plate (washed) 24 hour cultures of:

51 x 550.

o

typ.

(phage ??)

51 x 550 detected. + T.

Latent virus???? - no seenfu.

→ typical prototyp. colonies

42 x 70

42 x 37

= 42 x 61 (T.) -

13 x 36

1 x 45

42 x 45.

61 x 70 -

Trypan - many (++++)
do. v. small colonies

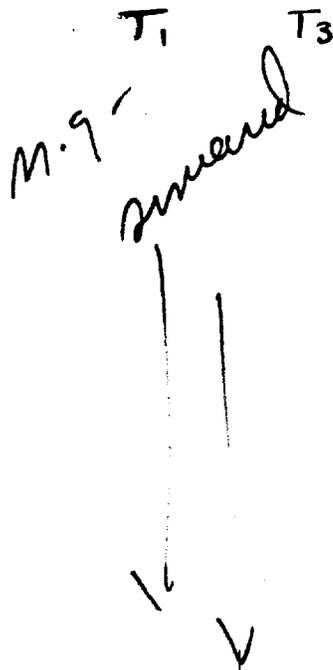
28001 1946

Plate 24 hr. cultures:

(A) Y10/1 + 58-161/3 only ca 5 colonies/plate

(B) Y10/5 + 58-161/1. ca 25 col./plate.

Y10/1
Y10/3
BM/1
BM/3



(A) (1)

(B) (2)

⋮

28 OCT 1946

Plate 24 hour cultures of: in-

$B^-T^-L^+$	0	*	$B^+T^+L^-$	0	10
$B^-T^+L^-$	0	0	0	0	400
3 strains:	0	100	1	0	

* same minute colonies

probably B^-T^+ on
agar bacteria

- picks minimum colonies for new stocks.

B^-L^+
 B^-T^- apparently only B^- appeared in the mixture. (from $B^-T^+L^- \times B^+L^+$)
must repeat + demonstrate recombination of all these strains!

Try $B^-L^- \times T^-L^-$ etc.But supports thesis of recom-
bination.

Y10: reversion.

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28 OCT. 1946

Plate washed Y10 in.

0

TL

ca 100 colonies.

TB₁

1 deep colony, n.g. for pic. long.

LB₁.

(plated too heavily.) ca 20 colonies.

Time of recombination.

29 OCT 1946

add 1ml old Y10 and Y41 into ca. 4ml YB. at various incubation times. At terminal time, wash all cultures + plate; compare prototroph frequency. Plate 5ml equiv. 30° sh. also compare YB + 5% glucose, YB + 5% NaCl, YB + 10% blood

24h.	1030 P 29.	1.	+++
12h.	10 A 30.	2	+++
10h.	12 N 30	3	+++
9h.	130 P 30	4	+++
7h.	330 P 30	5	+++
Centrifuge 1030 P.			
2h.	11 P.	6	+++
1h.	1215 A	7	++
1/2h.	1245.	8	-
Centr. 1245.			

The area - 2 hours. should be explored in more detail with comparative cell counts. Selective growth can not be a critical factor in so short a time. Also grow cells separately 1-2 hours + mix - hours + plate.

Conditions:

glucose	++	although growth was very much diminished!
NaCl	++±	
Blood	++	
" 1/100	-	
control	++	

29 OCT 1946

Sco.

S. cerevisiae haploid - (S.c.) on B(0) [Bunkeholder's \bar{E} 5% glucose].

V- 37° \bar{E} scaling preferable

B₁ - stimulatory (can youver??)

pab-

pyridoxine

pantothenate

inositol

biotin.

} required on this medium.

cf. Fries. (may contain B₆, inositol in the lactate.)

came up in 48 hours on wayfling but:

- biotin
- pab - pant.

6 vials above - O B₁ pab B₆ pant inos biotin
+++ +++ + +++ + ++ ±

- pant + alan + pntate
++ +

- pab + B₆
+

also Biotin Biotin pab Biotin Biotin Biotin V -pab
pab pab pant Biotin pntate pntate p-alamine +meth
pant pal pal pal pal pal pal + yna
+++ + ± ± + +++ +

can youver evidently too large for critical work; however, it is clear that Biotin and pab are essential; and β -alanine limiting.

S1 x S50.

342

1 NOV 1946

5 cultures each - S1, S50, S1 x S50. *more heavily*
P 1.

Plate N4 $\frac{1}{2}$ ml \approx or .01 ml \approx .

S1 - dil.

colonies appear which are the same in approx. number as those which occur in the S1 + S50 mixture. It is difficult to account for them on the basis of double reversion!

S50 - dil.
no colonies.

S1 + S50 - dil.

v. supra.

S1 x S50. - phage

3 4/3

Plate 3la cultures of S1 + of S50 as follows.
on nutrient saline agar.

S1 10^{-7} + S50. = S1/50 etc.

1. 1/50

2. 50/1

3. 1/1

4. 1/50

5. 1/1

6. 50/1

7. 1/1,50.

no evidence of phage action.

when area previously was picked, it showed
no ~~transmissible~~ transmissible lysis. Therefore this hypothesis is probably
not tenable !!

Recombination: Time of Occurrence

Y10 X Y40.

4 NOV 1946

Prepare fresh cultures for inocula. YP4.

Inoc 10 ml YB with 1 ml each inoc. at various times as indicated.
(Calculate from beginning of centrifuging) 28°.

Read initial and final optical densities to measure amount of growth.

Transfer cultures to smaller tubes for centrifuging and washing.
Inoc. 0.5 ml each washed culture into a minimal agar plate
Inoc. 1 ml into 10 ml prepared H₂O to measure the inoculum size.
Plan experiment to last 2 hours plus 1 hour for wash and plate

Time:	Initial d	Final d.	growth \times	Inoc. d. (cells)	colonies	$\text{P}/10^9$
* 1. 2h	10:07	66'	2882	1.60	ca 20.	10
		66 ²	2059	1.14	11	9
2. 1.5	10:37	66'	2596	1.44	20	10
		66 ²	2596	1.44	14	10
3. 1.0	11:07	65 ²	2291	1.27	ca 20	15
		64 ²	2366	1.31	18	15
4. .5	11:42	1800	2007	1.11	25	12
		62'	2059	1.14	14	11
5. 15m	11:57	64 ²	1905	1.05	20	20
		64 ²	"	1.05	—	—
6. 0.25	Mixed 12:10.	65	—	1.00	—	0
				—	25!	25!

83²: 660 filter.

* Mix immediately before washing; in 7. mix after washing.

Washing commenced at:

#4 on 540 filter.

Centrifuging 12:24.

15: 77
73²

Readings at 12:10

* contains Neurospora cont; inhibited around 1 colony cell 344-1 N. cont.
In this run, there seems to be an appreciable frequency of recombination as mixing in the plates!!!

Later: separate cells from medium before testing

Segregation of lactose fermentation.

5 NOV 1946

Prepare inocula P5 in modified YB broth: per liter -

Nutrient Broth	8
Yeast extract	1
R ₂ HPO ₄	3
KH ₂ PO ₄	1
NaCl	5
Glucose	10.

Plate after 4 hrs. growth.

a) ~~YB-glucose~~ NYB-glucose

b. NYB-lactose

c. NYB-no sugar.

all colonies are smaller
on lactose + than on glucose plates!

1. Y53 a x Y40 a in NYB-glucose.

Plate in: glucose -
lactose

187! v. variable!

247! v. variable size.

2. Y53 c x Y40 c in NYB -
glucose

no colonies.

lactose

no colonies.

3. Y53 a x Y40 b in NYB -
glucose

lactose

4. Y53 b x Y40 b in NYB - lactose
glucose5. Y53 a x Y40 a in NYB - lactose
glucose

lactose

6. Y53 b x Y40 c in NYB

no colonies

glucose

no colonies.

lactose

7. Y53 b x Y40 b in NYB.

glucose

118 do.

lactose

120. uniform size

	$L^+ T_1^R$	$L^+ T_1^S$	$L^- T_1^R$	$L^- T_1^S$	
7 gluc.	4 ✓	-	6 ✓	-	Test Lac ⁺ after replenishing on glucose medium.
lac	3 ✓	1 ✓	1 ✓	2 ✓	
4. gluc	3 ✓	1 ✓	4 ✓	2 ✓	
lac	2	-	7	2	
1 gluc	1	1	7	2	
lac	4 ✓		6 ✓		
	<hr/>		<hr/>		
	16	4	31	6	

	L^+	L^-
7+4: lac	5	12
gluc.	8	12
	<hr/>	<hr/>
	13	24
	4	6
	3	7
	<hr/>	<hr/>
	7	13

	L^+	L^-	
R	16	31	47
S	4	6	10
	<hr/>	<hr/>	<hr/>
	20	37	57
			35% L^+
	20% S	17%	$\chi^2 = 4.6$
			$p = .02$

18% S
 $\chi^2 = 2.3$
 $p < .001$

The presence of asparagine in the plating medium might be responsible for loss of fermentative ability and segregation of the gene. Must the media contain no other carbon source?? Grow cells on synthetic medium, 453 on glucose; 410 on lactose; note in presence of lactose only 5 growth; plate in lactose minimal (5 asparagine) and in glucose and compare numbers which appear; also test Lac⁺ for dissimilation.

Salmonella CROSSES
S42 x S45; S61 x S70.

346.

NOV 1946

In YB broz 11A5

Crosses YB 4PR

- dil.

S42

~~S45~~

v. numerous colonies.

S42 + S45

turbid dil

S61

S70

turbid. ^{numerous}
v. minute colonies.

x

turbid
no colonies.

Dissimilation of lactose fermenters

347

10 NOV 1946

Steels out fermenters from 345-42 and -72 on nutrient -
saline agar plates. Pitest isolates on EMB-lactose.

all as before. (4)

Non-Genetic; Nutritional Adaptation

Disproportionate insegregations can be interpreted on the basis of the transfer of cytoplasmic factors, or cytoplasmic centers. Since growth rate on minimal medium is less than on complete, the efficiency of plasmagenes in biosynthetic processes is a limiting factor and one expects that there will be, on minimal, selection for those cells which by virtue of essentially non-genetic variations have the most numerous and efficient cytoplasmic factors; on complete medium, there will be no selection at this level; if anything, it will be for efficiency at later synthetic steps (e.g. protein synthesis). This might be revealed by comparing cells grown on synthetic and on supplemented medium with respect to the lag which they exhibit when transferred, after washing, to minimal medium. Briefly, can cells become adapted to minimal medium? (in the adaptive enzyme sense, as opposed to gene mutation.)

For media use - coli (0) + 1% glucose; as a supplement, add vitamins, hydrolyzed yeast nucleic acid, and hydrolyzed casein.

Wash cells in coli (0) + glucose or asparagine or NH_4 source.
i.e. in phosphate buffer
 Na_2SO_4
 NaCl
trace of
 MgSO_4 ; CaCl_2

Nutritional adaptation

343a

11/7/46

- a. Large inoculum from a slant. Shake at 37° for 15 hours.
resuspend in 10 ml. water, ca. 0.2 ml.
- b. 1 ml. from a in same medium. (930H8) Shake 37°

ms.

- | | | |
|----|--------------------|--------------|
| 1. | lactose adapted a. | into lactose |
| 2 | | glucose |
| 3 | Glucose adapted a | into L |
| 4 | | G |
| 5 | α adapted a | into α |
| 6 | | 0 |
| 7 | 0 adapted a | into 0 |
| 8 | | α. |

11 etc. is as above c b.

4/4	4/6	6/2	6/5	4/10	0/0	0/10	0/0	Time	hours
1	2	3	4	5	6	7	8		
88	88 ³	92 ³	92	96	98	91		0	1045A
89	89	92 ³	91	95'	97 ³	91 ²	93 ²	30m.	1115A
89 ³	89 ²	93 ³	90	92 ²	97 ³	90'	94'	2h.	1245A
90'	90'	88	76 ²	94 ³	97 ²	59'	61'	4 1/4	3P
90 ³	90 ²	75'	67 ³	94 ³	98	38 ³	39'	5 1/2	420P
90'	91	62 ²	54'	94 ³	98	34'	34	6 3/4	530P
+	+		29	+	+		30		12M

log 2 1/2 hr log 1 hour

log 1 = 1 hour

(30 = 2 x 15 = 3)

11	12	13	14	15	16	17	18
			73	80'	80	80'	80

9 NOV 1946

Grow Y54 + Y41 in YB 1 1/2 days. Plate in T(0) p 9.

P12 - numerous yellow + white colonies (cont??) -

check for T_1^R , lac⁺.

W/ S

Segregation of Lac⁻; Evidence on transformation.

18 NOV 1946

6 PD prepare media.

Mix (1-2ml mix / 2ml YB) and
sh. 10A11. Wash + plate 1P.

1. ~~Y53~~ Y53 x 58-161
TLB, Lac⁻ T₁^S BM Lac⁺ T₁^R
2. ~~Y53~~ x 58-161
Y1011 TLB, Lac⁺ T₁^R BM Lac⁺ T₁^R
3. ✓ Y53 + Y1011 + 58-161.
TLB, Lac⁻ T₁^S TLB, Lac⁺ T₁^R BM Lac⁺ T₁^R
4. ✓ Y53 x Y40.
TLB, Lac⁻ T₁^S BM Lac⁺ T₁^R ^{in glucose} as usual.
5. ✓ Y53 x Y40 (lactose) in YB-lactose
plate in lactose-minimal; 2
and in glucose- " 6
(5 asparagine).

yellow colonies present in
all plates. Entenvariant some-
where!! (Wash. water????)

do not use, of course.

N.B.: If mixing of factors can take place (i.e. transformation) +++ should be found which are
Lac⁺ T₁^R (such as are found in case 4.)

Conditions for exp: fume and salt

9 NOV 1946

Y10; Y40. prepare inocula 1) P8.
separately 2) 1P8.

6 P8: inoculate 1.5 ml of mixture into YB + extending NaCl core. (4 wash
830P. wash 9P plate, .5 ml = into T(0) agar. 4B mix
d. 140c.

1	35.
2	60
3	35
4	20
5	27
6	13
7	5
8	5
9	10
10	4.

Best is to grow in
ca 2-3% NaCl
or to grow separately +
mix after washing +
use relatively young
inocula.

Also, inoculate @ 5% & separate cultures, 1ml. washes above
11- mix 0's in 5% and rewash. 20.

~~12- mix 0's after washing~~

12- mix 5's after washing. 30.

Effects of saline: Test for agglutination by salt of mixed culture -

5 hours

	aggl.	gc. nits.
0	-	-
1	-	-
2	++	-
3	++	-
4	++	-
5	++	-
6	+++	+
7	+++	+
8	+++	+
9	++++	++
10	++++	++

Sex conditions

352.

Wash 10 ml 16 hr. cultures (YB) of 440, 453 in water. 10 AM.

Mix 1 ml samples at various times; and dil part mixture \rightarrow 2.5% NaCl. plate at: 3 PM. Max (culture). shake gently at 30°.

	H ₂ O.	NaCl.
1. 10 A.M.	20.	6

Note: they were more dense in the H₂O than in NaCl

2. 11:30 A.M.	8	5
---------------	---	---

$\left(\frac{c_1}{c_2}\right)^2 = ca 4$. which might account for the results.

3. 1:30 P.M.	14	1
--------------	----	---

4. 3 P.M.	<u>2</u> plate.	<u>0</u> .
-----------	-----------------	------------

5. Mix 1 ml sep. culture in 5 ml agar + pour successively. 0.

Use younger cultures. No comparison of effectiveness of NaCl in increasing rate.

Segregation of lac^-

Repeat 350.

lysine inocula in YAG 3:30 P 14
 incubate in YAG 11A-2 P4 31.

1. Y53 - 58-161
 $T_1^S Lac^-$ or $T_1^S Lac^+$

Too few to pick

2. Y10/1 - 58-161
 $T_1^R Lac^+$ $T_1^S Lac^+$

Too few to pick.

3. Y53-Y10/1 - 58/161

11 $T_1^S Lac^-$ 4 $T_1^S Lac^+$

total $\left\{ \begin{array}{l} Lac^- 41 \\ Lac^+ 13 \\ \hline 54 \end{array} \right.$

no $T_1^R Lac^-$

4. Y53 - Y40. Most plates too smeared to be readable.

9 $T_1^R Lac^-$ 2 $T_1^R Lac^+$

3 $T_1^S Lac^-$

0 $T_1^S Lac^+$

5. Y54 - Y10/1

no colonies.

total $\left\{ \begin{array}{l} Lac^- 41 \\ Lac^+ 13 \\ \hline 54 \end{array} \right.$

= 24%

54

get Y53/1

See previous exps.

Phages on var. coli strains

		T1 ✓	T2 ✓	T3	T4 ✓	T5 ✓	T6 ✓	T7	φ-C
Y54	541, 542								
Y40	TTB, Lac:								
1	Y54	S	S ±	S ✓		S	S	S	S
2	Y40	R	S ±	R	<i>lots too low? plaque each m</i>	R	S	S	<i>S all lac-</i>
3	Y53	S	S ±	S		S	S ?	S	
4	58-161	S	S	S		S	S	S	
5	Y1011	R	S	R		R	S	S	
6	"B4/3"	R ✓	S	R		R	S	S	
7	"Y1013"	S ✓	S	S		S	S	S	

5) to Y1011, 3, 5 compare

plaque size small!!

OK. *later*

Throw out these "B4/3", "Y1013"

T1 = (R/1, 5)

plate phages + bacteria on surface of EMBO plates to secure other resistant types.

- ✓ Y1011/7 ca 10³
- ✓ 58-161/7 ca 10²
- ✓ Y53/3 ca 10²
- ✓ Y53/7 ca 10²
- ✓ Y40/7 smeared - ca 10³
- ✓ 58-161/3 ca 10³ smeared.
- ✓ Y1011/3 smeared - rather high titer - is Y1011 T3^S ??
- Y40/3 smeared. - do. Y40/3

K-12/1 ca 10^{3.5}

lost?

smeared

common said on 1st strain. like S.

do. " " " phage S

Phage Resistance groups.

Parent.	Phage.	T1	2	3	*	5	6	7	
Y53 M S K	T1	1 R	R	R	R	R		R	
		2 R	R	R	R	R		R	
		3 R	R	R	R	R		R	
		4 R	R	R	R	R		R	
		5 R	R	R	R	R		R	
		6 R	R	R	R	R		S ↓ ?	R
		7 R	R	R	R	R			R
		8 R	R	R	R	R			R
		9 R	R	R	R	R			R
		10 R	R	R	R	R			R
		11 R	R	R	R	R			R
		12 R	R	R	R	R			R
		13 R	R	R	R	R			R
		14 R	R	R	R	R			R
		15 R	R	R	R	R			R
		16 R	R	R	R	R			R
		17 R	R	R	R	R			R
		18 R	R	R	R	R			R
		19 R	R	R	R	R			R
		20 R	R	R	R	R			R
Y1011 microd	T7.	1 R ↓	not available	R ↓		R	not available;	R	
		2	do.			R	not free of phage		
		3	not available			R	resistant as a whole		
		4	do.			R	but entire stock has phage		
		5							
		6							
		7							
		8							
		9							
		10							
Y53 M	T7 ³	1 R	R	R	R	R	not available.	R	
		2 R	R	R	R	R		R	
		3 R	R	R	R	R		R	
		4 R	R	R	R	R		R	
		5 S	S ?	R	R	R		R	
		6 S	S ?	R	R	R		R	
		7 S	S ?	R	R	R		R	
		8 S		R	R	R		R	
		9 S		R	R	R		R	
		10 S		R	R	R		R	

resistance may be due to flow of microd growth??

resistant as a whole but entire stock has phage

should be checked!

Phage resistance groups

356

	T ₁	T ₂	3	5	6	7
Y53						
1			R	R		R
2				R		R
3				R		R
4				R		R
5				R		R
6				R		R
7				S		R
8				R		R
9				R		R
10				R		R

Y53 mucoid
E
T₁ ?
T₂ do.
all with latex (not for phage)
later non-mucoid
↓ with latex disrupt
no plaques (to mucoid?)
less mucoid.

Test isolates for streaks

	1	2	3	4	5	6	7	8	9
Y10/1	R	S	R	R	S	S	S	R	S
Y53	S	S	S	S	S	S	S	R	S
Y55	S	S	S	S	S	S	S	R	S
Y57	S	S	(S)=R	S-R	S	S	S	R	S
Y58	S	S	S	S	S	S	S	R	S
Y59	R	S	R	R	S	S	S	R	S
Y60	R	S	R	R	S	S	S	R	S
Y61	R	S	R	R	S	S	S	R	S
Y62	S	S	S	S	S	S	S	R	S

Y53/3
Y53/7
Y10/1/7
Y497
58-161/3
mucoid.
! - compare E. (mucoid).
plaques v. small.

The 13 resistants seem to become sensitive very rapidly when removed from phage!

	1	2	3	4	5	6	7	8	9	10
F=Y53/1	R									
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

all are 1, 3, 5

Phage resistance patterns.

357.

Conclusions.

- A. } all (smooth) T_1^R are also $T_3^R T_5^R T_2^S T_6^?$
B. } smooth T_1^R are resistant to all phages, incl. $T_2, T_7..$
F. }

- C. An fresh isolation, Y101; Y531 give T_7^R which contain phage, and
E. which are plaque infested, suggesting either virus mutations, or loss of resistance by bacteria.

on streaking out, Y53/7 has given rise to $Y53T_7^S$. (reversion?)
Y401/7; Y101/7 → $Y53T_7^R$ OK.

- D. Y53/3 shows equivoal resistance to $T_3; T_5$ after purification. K1.
1, 3, 5, 7. app. only less obt. on immediate streaking.

G.

Sex: conditions.

a) 36 hour hours cultures: Y53, Y40.

b) prepare fresh cultures from these 1130 A18. - compare when mixed in
 H_2O (after washing) for 1 hour. (see 352). Prepare the b) mixtures in
1:5 dilutions also. c.

a). 1ml eq. each in 1ml total

b.) do.

c.) as above + 4 ml. H_2O .

Plate comparable nos. of cells however.

d. 1:5 - a. 75
 b. 91
 1.5×10^9 $R=1.4$

∴ fresh cultures all settle there
 1) all

2) post incubation in H_2O is
 not possible.

3) dilution effect is questionable.

(ca 10^{-7}).

old	Time 0	Time 1h
A	.5 1.0	.5 1.0
	3 6	20

new B	90	22
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new del c 6

Test for significance:
 T_1^R lac - 19
 T_1^R lac + 2
 T_1^S lac - 3
 T_1^B lac + 0